Treatment of Renal Anemia with Roxadustat: Advantages and Achievement

Zuo-Lin Li Yan Tu Bi-Cheng Liu

Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, China

Keywords

Renal anemia · Roxadustat · Erythropoietin · Iron metabolism · Heparin

Abstract

Background: Although renal anemia has attracted widespread attention, a large proportion of chronic kidney disease (CKD) patients with anemia still do not meet the hemoglobin (Hb) targets. The discovery of prolyl hydroxylase domain (PHD) enzymes as regulators of hypoxia-inducible factor (HIF)-dependent erythropoiesis has led to the development of novel therapeutic agents for renal anemia. Roxadustat, the first small-molecule HIF-PHD inhibitor, has completed the phase 3 trials. There are currently more than 15 phase 3 clinical trials worldwide assessing the efficacy and safety of roxadustat in CKD patients with anemia. This review will summarize recent findings of roxadustat in the treatment of renal anemia. **Summary:** Although the administration of erythropoiesis-stimulating agents (ESAs) and iron supplementation are a well-established and highly effective therapeutic approach for renal anemia, there are several

safety concerns. Current findings from phase 2 and 3 trials suggest that roxadustat is clinically effective and well tolerated. On the one hand, roxadustat could increase endogenous erythropoietin (EPO) levels within or near physiological range in a titratable manner by inducing HIF pathway activation transiently. On the other hand, roxadustat also improves iron metabolism by decreasing serum hepcidin and increasing intestinal iron absorption, which is beneficial to functional iron deficiency and absolute iron deficiency. More importantly, the erythropoietic response of roxadustat is independent of baseline inflammatory state of CKD patients. Thus, the discovery of roxadustat will revolutionize the treatment strategy for renal anemia. **Key Messages:** Roxadustat is an emerging and promising therapeutic approach against anemia in CKD patients, which differs from those of conventional ESAs. Roxadustat corrects anemia of CKD patients through multiple pathways, beyond elevating EPO levels within physiological range, and also by handling iron metabolism (particularly decreasing the hepcidin levels). Furthermore, the Hb response of roxadustat is independent of the inflammatory microenvironment.

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Introduction

Since the link between uremia and anemia was first described by Richard Bright almost 200 years ago [1], anemia, which contributes to increased morbidity and mortality of patients with chronic kidney disease (CKD) [2], has become one of the most characteristic and visible manifestations of CKD [3]. Despite many advances in understanding of anemia, effective and safe treatment strategies are limited, and a large proportion of CKD patients with anemia still do not meet the hemoglobin (Hb) targets.

Mechanisms of Anemia in CKD

Renal anemia is typically characterized as normocytic, normochromic, and hypoproliferative. The data from clinical and preclinical studies suggested the etiology of anemia in CKD is multifactorial, including erythropoietin (EPO) deficiency, abnormal iron metabolism, blood loss, chronic inflammation, reduced erythrocyte survival duration, infection, oxidative stress, and nutritional deficits [4, 5], among which EPO deficiency is most predominant and specific. Findings from various genetic models provide convincing evidence that peritubular interstitial fibroblast-like cells (termed renal EPO-producing cells, RPCs) are the major source of EPO in the kidney [6, 7]. Dysfunction of RPCs, which transdifferentiated into myofibroblasts under conditions of CKD [8], leads to the loss of their ability to synthesize EPO, resulting in EPO deficiency and development of renal anemia. More interestingly, the evidence from genetic fate mapping studies supported the concept that the majority of myofibroblasts were derived from RPCs [9], providing an explanation for correlation with hematocrit, renal EPO mRNA, and renal fibrosis. Moreover, patients who benefited from the recombinant human EPO and related erythropoiesis-stimulating agents (ESAs) [10] also strongly supported the viewpoint that EPO deficiency is the most dominant cause of anemia.

Apart from EPO deficiency, negative iron balance is also a critical contributor to the renal anemia in CKD patients [11, 12]. Increased iron losses (approximately as much as 4–5 g iron per year) [13] and impaired dietary iron absorption (oral iron is no better than placebo and was less effective than intravenous [IV] iron at improving anemia) [14, 15] result in true iron deficiency. In addition, functional iron deficiency, characterized by impaired iron release from body stores, is another key factor

for renal anemia [16], manifested by a low transferrin saturation and high ferritin. It should be noted that there is still no effective therapeutic strategy for the management of these patients.

Increasing evidence indicates that hepcidin excess could provide the explanation for disordered iron homeostasis in many CKD patients [17]. It is now well established that hepcidin is the central regulator responsible for maintaining homeostasis of systemic iron. As the sole known exporter responsible for iron export into the plasma, hepcidin could maintain systemic iron balance via regulating the ferroportin, an iron channel on the surface of enterocytes, hepatocytes, macrophages, and placental cells [18]. It is recognized that hepcidin is regulated in response to the signaling pathways about inflammation, iron metabolism, hypoxia, and erythropoietic demand. The best-characterized mechanism is direct transcriptional activation of hepatic hepcidin expression via STAT3 pathway under the condition of inflammatory microenvironment (in particular inflammatory cytokines interleukin-6 [IL-6] and IL-1 β) [19]. Indeed, many patients with CKD have a chronic inflammatory state [20]. Recognition of the critical effect of hepcidin on iron metabolism and renal anemia has ignited interest in targeting hepcidin as a new treatment strategy [21].

Safety Concerns of Current Treatment Strategy for Renal Anemia

Currently, the use of ESAs and iron supplementation is the cornerstone of treatment of renal anemia. Although the administration of ESAs and iron supplementation are a well-established and highly effective therapeutic approach for renal anemia, there are several safety concerns. On the one hand, multiple trials of ESAs have demonstrated, at best, no improvement in outcome and, at worst, increased risks of cardiovascular events and mortality [22, 23]. On the other hand, due to the evidence of strong association between labile iron and oxidative stress, bacterial growth, severe gastrointestinal side effects, and hypersensitivity reactions, increased risks of infection and mortality have been concerns related to iron supplementation (in particular IV iron) in CKD patients [24, 25]. As suggested in a recent randomized trial, IV administration of iron is associated with an increased risk of serious adverse events, including cardiovascular and infectious diseases [26]. In fact, detailed concerns have been reviewed [27–29]. Thus, many other approaches have been explored to treat renal anemia [30].

Fig. 1. HIF coordinates erythropoietin production with iron metabolism. The oxygen-dependent PHDs as key regulators of HIF-dependent erythropoiesis became the direct therapeutic target for anemia correction. HIF-PHIs stabilize HIF via suppressing the function of PHDs directly. EPO synthesis could be stimulated by HIF (induced by pharmacologic PHD inhibition) in kidney and liver, which plays a central role in stimulating erythropoiesis in the bone marrow. In the duodenum, it is well recognized that DCYTB, DMT1, and FPN are regulated by HIF-2. DCYTB reduces Fe^{3+} to Fe^{2+} , which then enters enterocytes via DMT1. Iron is then re-

leased into the circulation via FPN. The increasing evidence demonstrated that HIF activation could suppress hepcidin expression, which increases FPN expression on enterocytes, hepatocytes, and macrophages, resulting in increased iron absorption and mobilization from internal stores. HRE, hypoxia response element; DCYTB, duodenal cytochrome b reductase 1; DMT1, divalent metal transporter-1; EPO, erythropoietin; EPOR, erythropoietin receptor; RBC, red blood cell; HIF, hypoxia-inducible factor; FPN, ferroportin; Fe^{2+} , ferrous iron; Fe^{3+} , ferric iron.

The Oxygen-Sensing Pathway and Roles of Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors

It has been well recognized that rapid induction of EPO production is one of the earliest and most sensitive physiological responses to hypoxia [31]. Actually, EPO synthesized in the kidney, which is the major source of circulating EPO, is regulated by renal oxygen concentration. Until 1992, discovering of hypoxia-inducible factors (HIFs) filled the gap in our understanding of hypoxia leading to erythropoiesis [32]. To date, the molecular mechanisms by which HIF pathway regulates erythropoiesis are well understood. The transcriptional activity of HIF is primarily controlled by its degradation rate, which is regulated by

oxygen sensors (known as prolyl-4-hydroxylase domain proteins, PHDs) [33, 34]. In addition to the EPO gene, HIF also targets molecules involved in multiple steps on iron absorption, metabolism, and transport [35]. Although it had been known since the 1980s that EPO transcription and HIF responses were activated with transition metals [36], the discovery of oxygen-dependent PHDs as key regulators of HIF-dependent erythropoiesis provided a theoretical basis for the development of HIF-activating compounds (called HIF-PHIs). Accordingly, recognizing the pivotal roles of the PHD-HIF axis in orchestrating erythropoiesis opened new avenues in the management of renal anemia [37, 38]. The target organs or tissues of HIF-PHIs for anemia correction are summarized in Figure 1.

Fig. 2. Key milestones in the development of roxadustat for the treatment of anemia caused by CKD. CFDA, China Food and Drug Administration; DD-CKD, dialysis-dependent chronic kidney disease; NDA, new drug application; US, United States; NDD-CKD, non-dialysis-dependent chronic kidney disease.

Roxadustat

Development History of Roxadustat

Roxadustat was developed almost a decade ago. Derived from its precursor (termed FG-2216), roxadustat (FG-4592), discovered by FibroGen, is a second-generation HIF-PHI, which differs from FG-2216 by the addition of a phenoxy substituent at carbon position seven of the quinoline core [39]. Accordingly, phase 1 clinical trials with roxadustat were initiated in November 2005. Based on the two phase 3 clinical trials in non-dialysis-dependent CKD (NDD-CKD) and dialysis-dependent CKD (DD-CKD) patients, roxadustat received its first approval in China on 17 December 2018 for the treatment of anemia in CKD patients on dialysis (including hemodialysis or peritoneal dialysis) [40]. On 16 August 2019, roxadustat received its approval for the treatment of anemia in NDD-CKD patients in China. Roxadustat has generated an encouraging opportunity for CKD patients by addressing a substantial unmet medical need in the treatment of renal anemia (Fig. 2). Here, we mainly reviewed the results of 3 clinical trials on roxadustat in CKD patients with anemia.

Roxadustat in Phase 2 Clinical Trials

The data from six phase 2 clinical trials published so far demonstrated that roxadustat could increase or maintain Hb levels of both non-dialysis and dialysis CKD pa-

tients (including ESA-naïve and ESA-converted patients) within the target range with good tolerability [41–46]. And more interestingly, it was demonstrated that roxadustat also had beneficial effects on iron metabolism (significant decreases in serum hepcidin levels particularly). Table 1 summarizes the Hb response and iron parameters in phase 2 clinical studies of roxadustat.

Moreover, roxadustat-induced anemia correction is independent of the degree of baseline inflammation state (assessed by serum C-reactive protein [CRP] levels). Furthermore, in addition to stimulating EPO production and regulating iron homeostasis, roxadustat (including other HIF-PHIs) has been shown to induce nonerythropoietic effects, such as lowering serum low-density lipoprotein or cholesterol levels in CKD patients [46, 47] and blood pressure-lowering effect in clinical or preclinical studies [42, 48]. Detailed results have been reviewed elsewhere [37, 38].

Roxadustat in Phase 3 Clinical Trials

Roxadustat is the first HIF-PHI entering the phase 3 trials. There are currently more than 15 phase 3 clinical trials and a target enrollment of about 10,000 patients worldwide studying the safety, efficacy, and long-term effects of roxadustat in CKD patients, including non-dialysis-dependent, hemodialysis-dependent, peritoneal dialysis-dependent, and patients on newly initiated dialysis [49]. And more importantly, ESA-naïve and ESA-con-

Table 1. Hb response and iron parameters in phase 2 trials

Identifier	Participants	Control	CFB of Hb	Iron parameters					Hepcidin
				CFB of ferritin	CFB of transferrin	CFB of TIBC	CFB of serum iron	CFB of TSAT	
NCT01414075	Incident HD and PD	Roxa	↑	↑	NS	↑	NS	↓	↓
NCT01147666	Maintenance HD	Roxa vs. epoetin alfa	↑	NS	NS	↑	NS	NS	NS
NCT01147666	Maintenance HD	Roxa vs. epoetin alfa	NS	NS	NS	NS	NS	NS	↓
NCT00761657	Non-dialysis-dependent CKD	Roxa vs. placebo	↑	↓	NA	↑	NS	↓	↓
NCT01599507 and 01596855	Non-dialysis-dependent CKD	Roxa vs. placebo	↑	↓	↑	↑	↓	↓	↓
NCT01964196	Non-dialysis-dependent CKD	Roxa vs. placebo	↑	↓	↑	↑	NS	↓	↓
NCT01244763	Non-dialysis-dependent CKD	Roxa	↑	↓	↑	NS	↓	↓	↓

CKD, chronic kidney disease; HD, hemodialysis; NA, not available; PD, peritoneal dialysis; NS, nonsignificant; Hb, hemoglobin; CFB, change from baseline; TIBC total iron binding capacity; TSAT, transferrin saturation; Roxa, roxadustat.

Table 2. Hb response and iron parameters in phase 3 trials

Identifier	Participants	Control	CFB of Hb	Iron parameters					Hepcidin
				CFB of ferritin	CFB of transferrin	CFB of TIBC	CFB of serum iron	CFB of TSAT	
NCT02652806	Maintenance HD or PD	Roxa vs. epoetin alfa	NS	NS	↑	↑	↑	↓	↓
NCT02652819	Non-dialysis-dependent CKD	Roxa vs. placebo	↑	↓	↑	↑	NS	↓	↓

CKD, chronic kidney disease; HD, hemodialysis; PD, peritoneal dialysis; NS, nonsignificant; Hb, hemoglobin; CFB, change from baseline; TIBC total iron binding capacity; TSAT, transferrin saturation; Roxa, roxadustat.

verted patients with various degrees of CKD were also included in those long-term phase 3 studies. Table 2 summarizes the Hb response and iron parameters in phase 3 clinical studies of roxadustat in patients from China.

Notably, two phase 3 trials regarding roxadustat for treatment of renal anemia carried out in China have recently been completed [50, 51]. In an initial 8-week, randomized, double-blind, placebo-controlled phase 3 trial (ClinicalTrials.gov NCT02652819), roxadustat was superior to placebo in increasing Hb levels in CKD patients

who were not undergoing dialysis. In this phase 3 trial, the mean $\pm$ SD CFB in the Hb level was an increase of 1.9 ± 1.2 g/dL in the roxadustat group and a decrease of 0.4 ± 0.8 g/dL in the placebo group ($p < 0.001$). Meanwhile, the study showed beneficial effects on iron metabolism (clinically stable serum iron levels, namely no significant difference between the two groups; increased in transferrin levels and total iron-binding capacity; decrease in transferrin saturation and ferritin levels). More interestingly, the reduction of hepcidin level from baseline was

56.14 ± 63.40 and 15.10 ± 48.06 ng/mL, respectively, for a between-group difference of -49.77 ng/mL (95% CI, -66.75 to -32.79).

In another randomized, open-label, active-controlled (epoetin alfa), phase 3 trial (ClinicalTrials.gov NCT02652806), evaluating the noninferiority of roxadustat, which was established if the lower boundary of the two-sided 95% confidence interval for the difference between the values in the roxadustat group and epoetin alfa group was greater than or equal to -1.0 g/dL, in patients undergoing long-term dialysis (at least 16 weeks), the results showed that roxadustat was not inferior to EPO (epoetin alfa) in increasing Hb levels. Although this trial comparing roxadustat with epoetin alfa for 26 weeks showed the noninferiority of roxadustat in the treatment of renal anemia, roxadustat treatment led to a numerically greater mean change in Hb level in CKD patients undergoing dialysis compared with those giving epoetin alfa treatment. Meanwhile, as compared with declined serum iron level in the epoetin alfa group, the serum iron level was clinically stable in the roxadustat group. It should be noted that in this trial, only the use of oral iron therapy was allowed. Actually, during the 26-week treatment period, 32.8% of patients in the roxadustat group received oral iron therapy, as compared with 43% in the epoetin alfa group ($p < 0.001$). Despite increased transferrin level and total iron-binding capacity, the decreased transferrin saturation supports the absorption-promoting effect on enteric iron with roxadustat. On the other hand, consistent with the results of previous phase 2 trials, hepcidin decreased significantly with roxadustat. Furthermore, the phase 3 trial of roxadustat involving patients undergoing hemodialysis addressed one important question, namely, the efficacy of roxadustat for CKD patients with an apparent inflammatory state (as assessed on the basis of CRP levels). Interestingly, among patients with elevated CRP levels, a greater increase in the Hb level response with roxadustat than those responses with epoetin alfa (despite receiving higher doses of epoetin alfa) was observed. Therefore, consistent with results in phase 2 studies of roxadustat, the results of phase 3 trials demonstrated that roxadustat could also improve anemia in CKD patients with inflammation. However, the detailed mechanisms are still not available. It is currently recognized that increased hepcidin expression in the liver, which leads to iron deficiency, could be induced by inflammation. Thus, the hepcidin-lowering effect of roxadustat could contribute to these findings in CKD patients with inflammatory state.

Recently, another phase 3 randomized, open-label, 24-week study (ClinicalTrials.gov NCT02780726) investigating the efficacy and safety of roxadustat in anemic Japanese patients on peritoneal dialysis who previously received ESA (ESA-converted group) or not (ESA-naïve group) has also been published [52]. Consistent with the results from the above two trials, roxadustat was effective in maintaining target Hb levels (92.3% in the ESA-naïve group and 74.4% in the ESA-converted group) in anemic patients on peritoneal dialysis. Meanwhile, the results also showed that iron metabolism parameters remained clinically stable throughout the study (only 1 patient was treated with IV iron), suggesting an increase in iron bioavailability via improving iron mobilization and absorption mediated by the observed reduction in serum hepcidin levels from baseline (also supported by the increase in transferrin). More importantly, roxadustat was generally safe and well tolerated in this study.

Advantages of Roxadustat

Roxadustat is an orally bioavailable, reversible HIF-PHI that mimics a novel way of utilizing the body's natural compensatory mechanisms in response to hypoxia, promoting HIF transcriptional activity. The data from trials illustrated that roxadustat corrects anemia of CKD patients with some distinct advantages.

One of the most striking superiorities is that roxadustat could induce a transient activation of HIF and increase in expression of HIF-regulated genes. With a half-life of approximately 12 h, roxadustat, administered twice or thrice weekly, enables HIF transcriptional activity to return to baseline between doses, which results in induction of expression of EPO in a titratable manner [53]. Furthermore, roxadustat transiently increased endogenous EPO levels within or near physiological range in patients with anemia of CKD. Thus, it appears that anemia correction with roxadustat could avoid these potential adverse effects caused by high ESA doses.

Beyond elevating EPO level, roxadustat could correct anemia by handling iron metabolism and particularly by decreasing the hepcidin levels, a response that was independent of baseline CRP levels and disease states of CKD patients (not receiving dialysis, hemodialysis, or peritoneal dialysis). This allows access to improve functional iron deficiency as well as increased absorption of oral iron, thus avoiding the use of high doses of IV iron, which has been associated with an increased inflammatory state and increased mortality [25]. The increase in transferrin

Table 3. Potential advantages of roxadustat based on clinical trials

Potential advantages of roxadustat
<ul style="list-style-type: none">– Increases or maintains Hb levels effectively– Increases endogenous EPO expression in physiological range– Regulates iron metabolism (hepcidin reduction in particular)– Increases iron absorption and is not influenced by inflammation– Oral route– Inhibits HIF-PHD reversibly and transiently– No risk of hypertension– Lowering of cholesterol levels– Avoidance of high EPO levels– Avoidance of side effects induced by iron supplementation
EPO, erythropoietin; Hb, hemoglobin; HIF-PHD, hypoxia-inducible factor-prolyl hydroxylase domain.

induced by roxadustat is a direct effect of HIF activation, as there are two HIF binding sites in the 5' enhancer region of the gene encoding transferrin [54]. However, it remains unclear how roxadustat is responsible for hepcidin suppression.

CKD anemia is also a type of chronic inflammatory anemia, which influences the erythropoiesis processes via inhibition of EPO production and disturbing bone marrow erythroblastic development and iron metabolism [55]. The data from phase 2 and 3 trials demonstrated that the erythropoietic response of roxadustat appears to be independent of inflammation, as correction and maintenance dose requirements were not associated with baseline CRP levels. This independence of response from the inflammatory state of patients implies that roxadustat might be one of the appropriate clinical strategies to treat anemia of CKD patients who have a hyporesponsiveness to ESA due to the high inflammatory state.

In addition, the decreased total cholesterol level was greater with roxadustat than with the control in phase 2 and 3 clinical trials. Meanwhile, compared with the epoetin alfa treatment, hypertension occurred at a lower frequency in patients with roxadustat, which has proved to be beneficial to cardiovascular events. The possible advantages of roxadustat are summarized in Table 3.

Potential Safety Concerns of Roxadustat

Although the data from phase 2 and 3 clinical trials in patients with CKD with or without dialysis have clearly demonstrated that roxadustat is effective and well toler-

ated, several important questions relating to the safety concerns need to be answered [56].

Accruing evidence has shown that HIF transcription factor directly regulates hundreds of genes, and consequently plays an important role in a broad spectrum of cellular functions and biological processes other than erythropoiesis, including energy metabolism, angiogenesis, mitochondrial metabolism, cellular growth and differentiation, inflammation, cell motility, matrix production, and epigenetics [57–59]. Therefore, it is reasonable to speculate that HIF stabilization by pharmacologic PHD inhibition will have a range of nonerythropoietic effects. Both phase 3 trials have shown a slightly higher risk of hyperkalemia with roxadustat than with control (epoetin alfa or placebo). In addition, the incidence of metabolic acidosis that was reported as adverse events was higher in the roxadustat group than in the control group (placebo) [51]. Furthermore, a potential proangiogenic effect related to the vascular endothelial growth factor (VEGF) and VEGF receptors, the possibility of development of pulmonary hypertension, and the potential progression of kidney disease related to the HIF activation over long periods are three intriguing questions [60, 61]. Accordingly, it is reasonable to await in-progress trials (ClinicalTrials.gov numbers NCT02052310, NCT02273726, and NCT01750190) and to proceed with additional studies reexamining the risks and benefits of normalization of the Hb level in patients with anemia of CKD and carefully monitoring for as yet unappreciated problems with roxadustat.

Conclusion

In summary, reduced EPO production and disorder of iron metabolism are two main characteristics of renal anemia. Roxadustat, as the first HIF-PHI, has been proven to exert an erythropoietic role in renal anemia just like one stone kills two birds by elevating the physiological range of endogenous EPO production and reducing hepcidin. In addition, roxadustat has been shown to successfully increase Hb level even with an inflammatory microenvironment, a status of CKD patients refractory to the current treatment of ESAs. It is thereby recognized that roxadustat could serve as a unique promising therapeutic approach against renal anemia differing from conventional ESAs. Although the data from phase 2 and phase 3 clinical trials indicate that roxadustat is an effective alternative for renal anemia with good tolerability, it should

be noted that more large-scale, long-term clinical trials for roxadustat in the treatment of renal anemia are still needed for a better understanding of its safety and non-erythropoietic effects.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Z.-L. Li and Y. Tu wrote the manuscript. B.-C. Liu supervised the work and revised the manuscript.

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